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Field Performance and Morphological Variations of X-Ray Induced M2 Mutant Lines of Fluted Pumpkin (*Telfairia Occidentalis*, Hook. F.)

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ABSTRACT

Fluted pumpkin *(Telfairia occidentalis*) is a vegetable widely cultivated for its nutritional value. The crop has narrow genetic variability which makes its improvement difficult. Induced mutagenesis through x-ray irradiation has been identified as one of the approaches to be adopted to create variability in this crop. Therefore the objective of this was to evaluate the field performance and morphological variation of fluted pumpkin treated with various doses of x-ray radiation at M_2generation. The x-ray doses used were 0kv (control), 40, 60, 80 and 100Kv. The irradiated seeds were planted on the field for the M_1V_1generation. The experiment was planted in randomized complete block design (RCBD), replicated three times. Data were collected on vine length, number of leaves, number of tendrils, number of primary branches, number of secondary branches and pod characteristics. Field observation of M_1 generation indicated that high doses of x-ray radiation reduce germination and the survival percentage. The LD_{50} (50% lethality) was obtained at 60Kv. All the morphological traits were also affected with increase in x-ray dosage. The number of fluted pumpkin pods produced followed the same trend of x-ray treatments. The control gave the highest pod yield of about 15 pods while seeds treated with 100Kv dosage of x-ray radiation produced only 2 pods. In M_2V_2 generation, the percentage germination and the survival rate also varied according to the dosage of the x-ray irradiation with non-uniform establishment count after planting. The decline in survival rate occurred as a result of persisted physiological injuries transferred from M_1V_1 to M_2V_2 generation. Observation on the growth pattern of M_2 mutants of the fluted pumpkin showed significant variation on the morphological traits. Positive effect of the x-ray irradiation was observed on the vine-length, number of secondary branches and pod characteristics. There was variation on the number of pods, pod size and color from one treatment to another. The coefficient of variability for the pod characteristics ranges from 21.1 - 29.90% as evidence of induced variation by the x-ray irradiation. The deviation in morphological traits of the mutants from the parental stock (control) was an indication of new creation of genetic variability in the irradiated fluted pumpkin.

Keywords: Fluted pumpkin, Improvement, Irradiation, Mutants, variability, X- ray.

INTRODUCTION

Fluted pumpkin (*Telfairia occidentalis,* Hook. F) is a vegetable in the family *cucurbitaceae* and is widely cultivated across lowland humid tropic of West African for its nutritional value. It is important in human and livestock nutrition and also serves as a cheap source of protein, oil and fat, vitamins and mineral (Taylor *et. al*, 1983). The seed contains about 21% protein and 13% fat (Tindal, 1975). The young shoots and leaves of this vegetable are sold for cash income by many small farm holders (Akorada, 1990). Despite the high nutritional value of fluted pumpkin and its importance, its production level hardly meets the demand required. The major problem of fluted pumpkin production is lack of improved varieties for planting and farmers rely solely on the landraces. Variability within the species of *Telfairia occidentalis* is low (Fayeum and Odiyi, 2012; Odiaka et al, 2008) and improved varieties of the crop are non-existent. Therefore genetic improvement of the crop is an imperative to enhance the production of this highly valued vegetable.

The improvement of crop plants depends to a large extent on genetic variability within the species. Over the years, man relied upon spontaneously occurring variants due to mutation for improved yield and quality of crop plants (Herper, 1999). Mutation is the fundamental source of heritable variation among species and induced mutation has been used widely and rapidly to produce new varieties of crop.

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Induce mutation has led to remarkable advances in crop improvement. Plant characters such as height, disease resistance, yields and nutritional qualities have been obtained through induction by mutagenic agents such as X – ray and gamma – ray (Iwo *et. al,* 2013and Simmond 1979). It offers the possibility of inducing desired attributes that either cannot be expressed in nature or have been lost during evolution. Induced mutation either by physical or chemical mutagen is practical approach useful in varietal development of vegetative propagated plants. Therefore induced mutation in fluted pumpkin through the use of x – ray irradiation will create desirable traits useful in Telfaria improvement. The purpose of this study was to evaluate the field performance and the morphological variation of fluted pumpkin treated with various dosages of x – ray radiation at M_2 generation.

MATERIALS AND METHODS

The experiment was conducted in the Faculty of Agriculture, Forestry and Wildlife Resource Management, University of Calabar, Cross River State, Nigeria. Calabar is located in the tropical rainforest ecological zone of Nigeria. It lies at latitude 4^0 96 N of the equator and longitude 8^0 31 'E with bimodal annual rainfall ranging from 2000mm to 3000mm and average monthly temperature of 24^{oc} to 32^{oc}.

In 2012, forty seeds (M_o) of fluted pumpkin were irradiated using different doses of x – ray measured in kilo voltage pressure from x – ray machine source. The x – ray dosages were 0, 40, 60, 80 and 100kv. This was carried out at the University of Calabar Teaching Hospital, Calabar. The treated seeds were planted on the experimental field at University of Calabar Research farm for M_1V_1 generation. The land was cleared, ploughed and harrowed with tractor and each treatment was planted on 2 rows plot of 5m x 1.8m with inter –row spacing of 120cm and intra – row of 60cm without replication. Manual weeding was carried out two weeks after planting followed with hand weeding at two weeks interval. Fertilizer (NKP 15:15:15) was applied four weeks after planting using ring method of application.

In 2013 cropping season all the harvested M_1V_1 seeds were planted on the field for the M_2V_2 generation using Randomized Complete Block Design (RCBD) replicated three times. Seeds from each pod derived from each treatment was planted using Single Pod Descent (SPD) method. A plot size of 5m x 1.8m ($9m^2$) with inter – row of 120cm and intra - row of 60cm was maintained, and mulching was carried out immediately after planting to avoid rodent or lizards removing the seeds. All the cultural practices such as weeding and fertilizer application were carried out at3WAP and 5WAP respectively. The M_2V_2 plants were evaluated individually according to the treatments. Five plants per treatment within a plot were randomly selected for data collection. Data were collected on percentage germination, relative survival, vine length, number of tendrils/plant, number of secondary branches, pod size and pod weight. The relative survival and lengthy (LD50) were calculated as describe by Nwachukwu *et. al* (1994) and data on morphological traits were analyzed using mean and standard error.

$$\text{Relative Survival} = \frac{\text{Survival of dose treatment}}{\text{\% Survival of control}} \times 100$$

Lethality was estimated as: 100 – relative survival %.

The LD50 dose of irradiation was determined by using the observations on sprouting percentage and survival percent.

RESULTS AND DISCUSSION

Field performance of the M_1V_1 generation showed that the percentage germination of the seeds, survival rate and establishment count decreased with increased dose of x-ray while lethality increased with high doses of x-ray radiation. This support the observation by Dutta (1981) that the effect of mutagenic agents on plants materials are proportional to dosage and high dosage lead to chromosomal alteration and genetic changes. The LD50 (50% Lethality) was obtained at 60kv. High percentage germination of 86%was record at 40kv and 73% at 60kv while the percentage of survived plants at harvest, number of pods produced followed the trend of x-ray treatments. All the morphological traits were also affected with increase in x-ray dosage. The control (untreated) yielded 15 pods while seeds treated with x-ray irradiation produced 11 pods at(40Kv) ,7 pods(60Kv), 5 pods(80Kv) and 2 pods(100Kv) accordingly (Fig 1 and 2).

Physical mutagens has three types of effect; physiological damage (primary injury), factor mutation (Gene mutation), and Chromosome mutation (Chromosome aberration) (Thohiruh el al 2009). It is the physiological injury that reduces germination, survival ability, reduces number of leaves, pod formation and pod sizes. According to Iwo et al (2013), gene and chromosomes mutation due to irradiation can be transferred from M_1V_1 generation to M_2V_2 or subsequent ones.

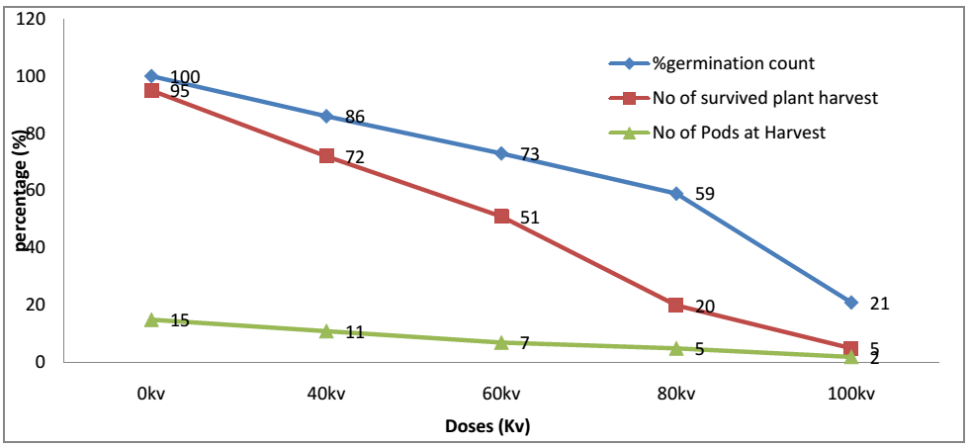

Fig1. *Radio sensitivity of fluted pumpkin seeds exposed to different doses of X-Ray*

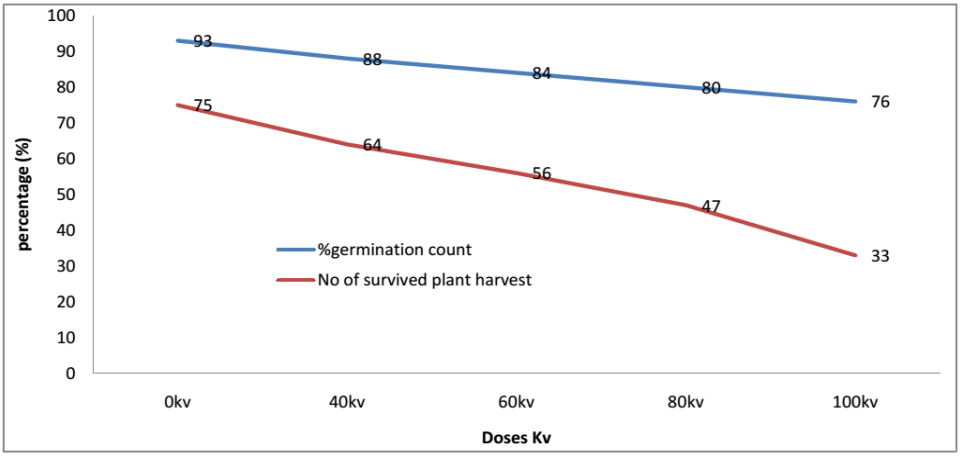

Fig2. *Percentage germination and survival fluted pumpkin seeds at M2V2*

In M_2V_2 generation, the percentage germination varied according to the dosage of the x-ray irradiation. The untreated (OKV) recorded the highest percentage germination of 93% while 100kv recorded 76%. Similar results were reported by Ahman and Qureshi (1992) in *Zea mays* L and Din *et. al* (2003) in *Triticum aestivum* L. There was variation in number of plants that survived and consequently the establishment after planting. The untreated (0kv) had 75 % survival followed by 40kv with (64%) survival and then 60kv (56%), 80kv (47%) and 100kv with (33%) respectively (Fig 2). The decline in survival rate occurred as a result of persisted physiological injuries transferred from M_1V_1 to M_2V_2 generation. The primary injuries retard or inhibit cell division and changes in plant morphology. At harvest less number of plants was recorded on treatments with higher doses as a deviation from the establishment count. Positive effect of the x-ray irradiation was observed on the morphological traits of the fluted pumpkin such as vine length, number of secondary branches and pod characteristics (Table 1). The general observation of the M_2 mutants was the variation between the irradiated plants and the control (untreated plant). The vine length was affected according to the dosage of the x – ray radiation. The vine length of untreated plant was 146cm while the vine length of irradiated plant with 100kv reduces to 116cm. Vine length is a major leave yield component of fluted pumpkin. It is believed that longer vine length yields higher number of leaves per plant. Contrary to this observation, there was no significant difference in number of leaves per vine in all the treatments except at 60kv, which gives 47 leaves/vine, a little above untreated plant. This shows that the leaves yield can be improved through irradiation at 60kv. There was also no significant difference in number tendril and primary branches among the treatment. The irradiation by x – ray seem to have no effect on these two components. Tendril is not a yield component to fluted pumpkin, but has supportive role

to the vines. There was significant variation on the number of secondary branches. The seeds treated with 40kv and 60kv gave higher number of secondary branches while seeds treated with 100kv gave rise to plants with fewer branches when compared with those irradiated with 40kv, 60kv, 80kv and the untreated (control). This shows that x – ray irradiation stimulated the formation of secondary branches which is a major leave yield component in fluted pumpkin. Variants were also observed on number of pods. The structure and color of the harvested pods from the plants irradiated with 40kv and 60kv were clearly different from the parent stock (control) (Plate. 1)

Plate1. *Pod characteristics of fruited pumpkin at different dosage of X-ray radiation.*

Table1. *Morphological traits variation at different doses of x – ray radiation for M2V2 pod generation.*

Treatment dosage (KV)	Vine Length Cm	Number of Leaves	Number of Tendrils	Number of Primary Branches	Number of Secondary Branches
0	146±6.1	46.8±1.8	11.3±0.5	2.3 ±0.1	4.7±0.4
40	132±5.8	42.7±3.0	11.1±0.6	2.0 ±0.0	5.1±0.6
60	120±5.4	47.6±2.8	11.9±0.6	2.0 ±0.4	4.9±0.2
80	118±6.3	43.4±1.8	10.0±0.6	2.1 ±0.2	3.0±0.5
10	116±5.4	40.5±1.8	10.2±0.5	1.2 ±0.1	2.9±0.2

The size of the pods decreased with increase in x – ray radiation dosage. The range of coefficient of variability of the pod characteristics from 21.1% - 29.90% is evidence of existing variation induced by the irradiation (Table 2). The deviation from the parent is also an indication of the effect of the mutagen. According to Brunner (1995) induced mutation play very vital role in altering the genetic make-up of genotypes not only at a chromosomal, but even at a molecular level. These result to creation of new genetic variation within the crop varieties.

Table2. *Mean values of the pod characteristics*

Treatment (KV)	Number of pods per treatment	Pod length (cm)	Weight of two pods (kg)	Circumference of the pod (cm)
0	5.00	17.50	6.10	24.60
40	3.00	18.20	6.00	21.60
60	2.00	17.95	4.00	20.95
80	4.00	15.65	2.00	16.35
100	3.00	10.30	0.40	13.00
Mean	3.40	15.92	3.68	19.34
SE±	0.46	1.90	0.99	1.82
CV (%)	29.90	20.70	27.10	21.2

CONCLUSION

The observation of field performance and variation in morphological traits of M_2 mutant lines of fluted pumpkin revealed the effectiveness of the induced mutation. The variation at different treatment levels of x-ray irradiation at M_2 mutative generation is linked to gene effect rather than environmental

and it will be useful for selection. At this stage, selection can be initiated but stability and fixation of genes or chromosomes due to alteration by x-ray irradiation may not be possible at this early stage. The morphological traits and pod characteristics of the irradiated fluted pumpkin plants at different dosage has indicated that the x-ray irradiation was effective in creating the variability which is an essential component in crop improvement. It is therefore recommended that the mutant lines be advanced to M_3V_3 or M_4V_4 if effective and reliable selection is to be achieved. At these generations the structural changes in genes will be stable and become fixed.

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